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(54) Title: METHOD FOR REMOVING HEAVY METALS FROM BONE

(57) Abstract

The present application discloses a method for removing heavy metal from a subject's bone tissue. The method includes contacting a subject's bone tissue with the subject's blood under conditions effective to transfer at least some of the heavy metal in the subject's bone tissue to the subject's blood. The blood fluid is extracorporeally circulated, and, while the blood is being extracorporeally circulated, at least a portion of the heavy metal is removed from the subject's blood. The method of the present invention can be used to decrease the level of heavy metal present in a subject's bone tissue and, in this manner, reduce a heavy metal pool which is believed to contribute to elevated blood levels of heavy metals with its associated physiologically damaging effects.

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METHOD FOR REMOVING HEAVY METALS FROM BONE

FIELD OF THE INVENTION

The subject invention relates, generally, to a method for removing a metal from bone and, more particularly, to a method for removing lead from bone.

BACKGROUND OF THE INVENTION

Lead intoxification, in recent years, has become a problem of such magnitude to create a medical emergency. In a recent government report, it was estimated that 230,000 children in the United States are in need of treatment for lead poisoning. The report also indicated that 400,000 pregnant women have lead levels around 15 μg/dL, a level safe for adults (by present standards of the Occupational Safety and Health Administration ("OSHA")) but found to be neurotoxic to the fetus. It has been concluded that, unless lead is removed from these women, a large number of children will reach school age with neurological handicaps. Further, OSHA has reported that the number of industrial workers with toxic lead levels is in the range of 500,000.

The major causes of lead intoxification are environmental and occupational exposure. Although much had been done to reduce these exposures using preventative measures, the population that already has lead poisoning needs treatment with a safe and effective method.

Several chelators are presently used to treat lead poisoning. These include $CaNa_2EDTA$ and BAL, which are administered by injection, and penicillamine and mercaptosuccinate, which are administered orally. These chelators have a variety of undesirable side-effects, such as renal toxicity when eliminated as a lead complex. Another method, which involves the use of a cartridge containing immobilized chelator for the extracorporeal removal of lead, has been described in U.S. Patent No. 4,612,122 to Ambrus et al.

However, these methods only relate to the removal of lead from blood when blood lead levels are greater than a threshold value. For example, children with lead poisoning are treated, mostly with $CaNa_2EDTA$, only at blood lead levels of greater than 15 μg/dL. Chelation therapy is mandated only at blood lead levels in excess of 45 μg/dL.

In children and in acute intoxications, the blood lead level is a good indicator of recently absorbed lead. Further, in these cases, it is a good indicator of the biologically active lead concentration that is responsible for toxic effects. However, lead, especially in cases of prolonged intoxication, becomes stored in various tissues of the body. Although lead stored in the soft tissues (e.g., kidney and liver) is chelatable and affects blood lead levels, for lead stored in the skeleton, chelation therapy has not generally been viewed as a practical method for removing lead from bone. Therefore, although treatment of subjects having high blood lead levels is viewed as critical in acute lead poisoning, the presently available methods fail to address the problems associated with lead distributed in various body tissues, particularly lead distributed in the skeleton.

Accordingly, there exists a need for a method of removing lead and other heavy metals from bone tissue. The present invention is directed to meeting this need.

SUMMARY OF THE INVENTION

The present invention relates to a method for removing heavy metal from a subject's bone tissue. The method includes contacting a subject's bone tissue with the subject's blood under conditions effective to transfer at least some of the heavy metal in the subject's bone to the subject's blood. The subject's blood is extracorporeally circulated, and at least a portion of the heavy metal is removed from the subject's blood while the blood is being extracorporeally circulated.

The method of the present invention can be used to decrease the level of heavy metal present in a subject's bone tissue and, in this manner, reduce a heavy metal pool which is believed to contribute to elevated blood levels of heavy metals with its associated physiologically damaging effects.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to a method for removing heavy metal from a subject's bone tissue. The method includes contacting a subject's bone tissue with the subject's blood under conditions effective to transfer at least some of the heavy metal in the subject's bone to the subject's blood. The subject's blood is extracoropreally circulated, and at least a portion of the heavy metal is removed from the subject's

blood while the blood is being extracoropreally circulated.

As used herein, "metal" is meant to include metal atoms (i.e., zero valent metal), metal ions, metal complexes (which can be neutral, cationic, or anionic), metal ligands (e.g., metal interacted with small proteins or polypeptides), metal hydrates, metal compounds (e.g., where a metal ion is ionically bound with one or more counterions), and the like. Examples of heavy metals which can be removed from bone tissue using the method of the present invention include cadmium, lead, and other metals whose elevated levels have adverse physiological effects. "Heavy metal", as used herein, are also meant to include combinations of these metals.

"Bone tissue", as used herein refers to any and all of the various types of bone found in the skeletal system. These include cortical bones, sponge bones, and trabecular bones.

"Removing heavy metal from a subject's bone tissue" is meant to include a partial as well as a total removal of heavy metal from the subject's bone tissue. Preferably, the amount of metal removed from the subject's bone tissue is sufficiently great to noticeably reduce (e.g., reduce by about 1%, 5%, and/or 10%) the level of heavy metal in subject's blood which derives from heavy metal stores in the subject's bone tissue.

The present invention can be used to remove lead and other heavy metals from the bone of a variety of subjects. These subjects include those who have elevated levels of heavy metal (e.g., lead). Suitable subjects also include those who have had elevated blood levels of heavy metal (e.g., lead) in the past, as determined, for example, by past medical records showing prior elevated

blood levels of heavy metal (e.g., lead). For example, the subject can be one who had previously undergone intervention (e.g., chelation therapy) to bring blood heavy metal (e.g., lead) levels to within normal ranges and who, as a result of this intervention presently has blood heavy metal (e.g., lead) levels below that which would mandate continued or additional removal of heavy metal (e.g., lead) from the blood. Alternatively, the subject can be one who has never been diagnosed as having elevated blood heavy metal levels and who, presently, has a blood concentration of heavy metal below those which would mandate intervention. The concentration of heavy metal in the blood which mandates intervention depends on the characteristics of the subject (e.g., age, sex, pregnancy status) as well as the identity of the heavy metal. These values are set by various governmental agencies, such as, OSHA. For example, adult, non-pregnant, human subjects having blood lead levels below about 45 μg/dL are viewed as not requiring intervention (e.g., chelation therapy) to reduce blood lead concentration. Other examples of adult subjects who are suitable candidates for the practice of the method of the present invention are those having blood lead levels below about 40 μg/dL, below about 35 μg/dL, below about 30 μg/dL, below about 25 μg/dL, below about 20 μg/dL, and/or below about 15 μg/dL. Children having blood lead levels below about 15 μg/dL are viewed as not requiring intervention (e.g., chelation therapy) to reduce blood lead concentration. Other examples of child subjects who are suitable candidates for the practice of the method of the present invention are those having blood lead levels below about 12 μg/dL, below about 10 μg/dL, below about 8 μg/dL, and/or below about 5 μg/dL. In particular, those

subjects who have blood lead levels below one or more of these values and who have previously had elevated blood lead levels (e.g., above about 45 μg/dL for adults and above about 15 μg/dL for children) are especially well situated to benefit from the present invention. Still other subjects particularly well situated to benefit from the practice of the present invention are those who have blood lead levels below one or more of these values, but who have had a history of prolonged exposure to elevated levels of heavy metal or whose exposure to elevated levels of heavy metal occurred more than ten years ago. Information regarding a subject's prior history regarding lead or other heavy metal exposure can be determined from past medical, work, and residence records and/or from interviews with the subject. Subjects who have blood lead levels below one or more of these values and who have elevated bone heavy metal concentrations (e.g., above about 10 μg/g, above about 15 μg/g, above about 20 μg/g, and/or above about 25 μg/g μg/g of bone) are especially well situated to benefit from the practice of the present invention.

A variety of methods can be used to measure the concentration of heavy metal in blood and bone samples. These include, for example, graphite furnace atomic absorption spectrometry, and x-ray fluorescence ("XRF") spectroscopy . Methods for preparing and handling these samples are provided, for example, in Rabinovitz, "Toxicokinetics of Bone Lead," Environmental Health Perspectives, 91:33-37 (1991); in D'Haese et al., "Aluminum, Iron, Lead, Cadmium, Copper, Zinc, Chromium, Magnesium, Strontium, and Calcium Content in Bone of End-stage Renal Failure Patients," Clinical Chemistry, 45:1548-1556 (1999); and in Smith et al., "Use of

Endogenous, Stable Lead Isotopes to Determine Release of Lead form the Skeleton," Environmental Health Perspectives, 104(1):60-66 (1996) ("Smith"), which is hereby incorporated by reference. The use of graphite furnace atomic absorption spectrometry is described, for example, in Smith, which is hereby incorporated by reference; and the use of XRF spectroscopy is described, for example, in Hu et al., "Bone Lead as a Marker in Epidemiologic Studies of Chronic Toxicity: Conceptual Paradigms,"Environmental Health Perspectives, 106:1-8 (1998), which is hereby incorporated by reference.

As indicated above, the heavy metal which can be removed from bone tissue in the practice of the present invention include cadmium and lead. In the case where the heavy metal in bone tissue is lead, the method can be used to remove the lead, irrespective of its isotopic composition. For example, the isotopic composition of the lead in bone can have a $^{207}Pb/^{206}Pb$ ratio which is substantially the same as that found in the subject's exogenous environment. Likewise, the bone tissue of the subject can contain lead having a $^{208}Pb/^{206}Pb$ ratio which is substantially the same as the $^{208}Pb/^{206}Pb$ ratio in the subject's exogenous environment. Alternatively, one or both of these ratios can be different than those found in the subject's exogenous environment. For example, the subject's bone tissue can contain lead having a $^{208}Pb/^{206}Pb$ ratio greater than 110% of the $^{208}Pb/^{206}Pb$ ratio in the subject's exogenous environment, or the subject's bone tissue can contain lead having a $^{207}Pb/^{206}Pb$ ratio greater than 110% of the $^{207}Pb/^{206}Pb$ ratio in the subject's exogenous environment, or both. Typically, when a subject's bone tissue contains $^{208}Pb/^{206}Pb$ and/or $^{207}Pb/^{206}Pb$ ratios greater than those in

the subject's exogenous environment, the lead in the bone tissue is the result of a prolonged exposure to elevated levels of lead or an exposure to elevated levels of lead more than ten years ago. The $^{208}Pb/^{206}Pb$ and/or $^{207}Pb/^{206}Pb$ ratios in a subject's bone tissue can be determined from a bone biopsy sample taken from the subject by methods, such as inductively-coupled plasma mass spectrometry ("ICPMS") or thermal ionization mass spectrometry ("TIMS") which have been previously described (see, e.g., Yoshinaga, "Isotope Ratio Analysis of Lead in Biological Materials By Inductively Coupled Plasma Mass Spectrometry," Tohoku J. Exp. Med., 178:37-47 (1996) ("Yoshinaga"); Delves et al., "Measurements of Total Lead Concentrations And of Isotope Ratios in Whole Blood By Use of Inductively Coupled Plasma Source Mass Spectrometry," J. Anal. Atom. Spectr., 3:343-348 (1988) ("Delves"); and Smith, which are hereby incorporated by reference).

Bone $^{208}Pb/^{206}Pb$ and/or $^{207}Pb/^{206}Pb$ ratios that are different than those of a subject's exogenous environment generally manifest themselves in blood $^{208}Pb/^{206}Pb$ and/or $^{207}Pb/^{206}Pb$ ratios that are different from than the subject's exogenous environment. A subject's blood $^{208}Pb/^{206}Pb$ and/or $^{207}Pb/^{206}Pb$ ratio that is different than those found in the subject's exogenous environment tends to indicate that the subject has a reserve of lead in bone tissue which is being slowly released into the blood, thus causing the blood $^{208}Pb/^{206}Pb$ and/or $^{207}Pb/^{206}Pb$ ratios to differ from the $^{208}Pb/^{206}Pb$ and/or $^{207}Pb/^{206}Pb$ ratios in the environment. For example, the subject's blood can contain lead having a $^{208}Pb/^{206}Pb$ ratio greater than 110% of the $^{208}Pb/^{206}Pb$ ratio in the subject's exogenous environment, or the subject's blood can contain

lead having a $^{207}Pb/^{206}Pb$ ratio greater than 110% of the $^{207}Pb/^{206}Pb$ ratio in the subject's exogenous environment, or both. In these cases, the increased $^{208}Pb/^{206}Pb$ and/or $^{207}Pb/^{206}Pb$ ratios in the blood indicates that the subject's bone tissue contains increased $^{208}Pb/^{206}Pb$ and/or $^{207}Pb/^{206}Pb$ ratios, which, as discussed above, is a sign that the lead in the bone tissue is the result of a prolonged exposure to elevated levels of lead or the result of an exposure to elevated levels of lead which occurred some time ago (e.g., more than 10 years ago, more than 17 years ago, more than 25 years ago, and/or more than 30 years ago). Accordingly, the method of the present invention is particularly well suited to subjects whose blood contains lead having a $^{208}Pb/^{206}Pb$ ratio greater than 110% of the $^{208}Pb/^{206}Pb$ ratio in the subject's exogenous environment, to subjects whose blood contains lead having a $^{207}Pb/^{206}Pb$ ratio greater than 110% of the $^{207}Pb/^{206}Pb$ ratio in the subject's exogenous environment, or to subjects who meet both of these conditions. Subjects who meet either or both of these conditions with regard to blood $^{207}Pb/^{206}Pb$ and/or $^{208}Pb/^{206}Pb$ ratios and who also have blood lead concentrations below those concentrations which would mandate intervention with, for example, chelation therapy (e.g., below about 45 μg/dL, below about 40 μg/dL, below about 35 μg/dL, below about 30 μg/dL, below about 25 μg/dL, below about 20 μg/dL, and/or below about 15 μg/dL), are especially well positioned to benefit from the practice of the present invention.

Methods for measuring the $^{207}Pb/^{206}Pb$ and/or $^{208}Pb/^{206}Pb$ ratios in blood can be carried out using a sample of the subject's blood by standard methods, such as by inductively-coulpled plasma mass spectrometry ("ICPMS") and thermal ionization mass spectrometry

("TIMS"), which are described, for example, in Smith, in Delves, and/or in Yoshinaga, which are hereby incorporated by reference.

As indicated above, the method of the present invention involves contacting the subject's bone tissue with the subject's blood under conditions effective to transfer at least some of the heavy metal in the subject's bone tissue to the subject's blood. Typically, the subject's own circulatory system is used to contact the subject's blood with the subject's bone tissue. It is believed that heavy metal is partitioned between blood and bone tissue according to an equilibrium. If the equilibrium is disturbed, heavy metal leaves the blood and enters the bone tissue (e.g., at times when the concentration of heavy metal in blood is high) or heavy metal leaves the bone tissue and enters the blood (e.g., at times when the concentration of heavy metal in the blood is low). Thus, the contacting is preferably carried out with a blood having a heavy metal concentration below that which was present when the equilibrium was established to ensure transfer of heavy metal from bone to the blood.

The subject's blood is extracorporeally circulated. As one skilled in the art will recognize this can be carried out by diverting blood flow from any portion of the subject's circulatory system, e.g., a vein or an artery. Typically, the subject's blood will be extracorporeally circulated, and the extracorporeal circuit will contain only a small fraction (e.g., < 10%) of the subject's total volume of blood.

At least a portion of the heavy metal is removed from the subject's blood while it is being circulated extracorporeally. This can be done in a

variety of ways. One particularly effective method for removing heavy metal ions from blood is described in U.S. Patent No. 4,612,122 to Ambrus et al., which is hereby incorporated by reference.

Briefly, blood to be purified is passed over an anisotropic membrane which is in intimate contact on the non-blood-wetted side of the membrane with an metal-capturing material, such as a non-proteinaceous chelating agent which accepts and immobilizes heavy metals. Typically, the chelating agent is not bound to the membrane.

The membrane can have very small pores, e.g., pores having a nominal molecular weight cut-off of less than 50,000. Such pores have effective pore sizes of only about 0.001-0.002 microns. The heavy metal finds its way through the pore sites to a region (e.g., an extra-fiber space), which phenomenon is best described as a convection-diffusion process, where the immobilized chelator will be able to seize the heavy metal. This. It does not require a liquid flow in the extra-fiber space for carrying the heavy metals into proximity to the chelator. Any liquid outside the membrane is substantially static and functions only as a diffusion path for the heavy metals.

The membrane is preferably an anisotropic membrane with the tight or retention side facing the bloodstream. Such membranes are most commonly used in ultrafiltration processes and are commercially available. The membrane is conveniently formed of any number of polymers known to the art. The degree of blood compatibility of the membrane should be high, so that it interacts favorably with blood.

One way of further increasing the efficacy with which the chelating resin is in contact with the membrane is to circulate a slurry or solution of the chelating agent rather than have a relatively static mass in contact with the membrane. It is also possible to utilize liquid chelating macromolecules that cannot pass through the membrane that are in static or dynamic contact with the opposite side of the membrane from the blood being processed. However, in many cases, the immobilized chelate is so efficient that there is little additional value to employing this recirculation technique.

It has been found that, even though some quantity of the heavy metal in the blood will be carried on organic molecules which are large enough to be retained in the bloodstream, a significant fraction of the heavy metal is in equilibrium with smaller molecules. As these smaller molecules (or free heavy metal) permeate the membrane and the heavy metal is captured by the chelating agent, the equilibrium reestablishes itself to assure the availability of still additional heavy metal to the chelate. This procedure continues until the concentration of heavy metal in the blood is substantially reduced, e.g., reduced to a level below the level at which treatment is mandated. Preferably, the concentration of heavy metal in the blood is reduced significantly below the level at which treatment is mandated (e.g., to less than about 50% and/or less than about 25% of the level at which treatment is mandated), so that, when contacted with the bone tissue, the equilibrium between bone and blood strongly favors transfer of heavy metal from bone to blood.

Suitable metal-capturing materials include organic chelating agents which, preferably, are immobilized on a larger structure, such as a resin. Multi-valent chelating agents, particularly multi-valent carboxylate salts, are preferred for use in the above-described preferred embodiment of the present invention. Multi-valent carboxylate salt-bearing resins are particularly useful as metal-capturing materials. Finely-divided chelating ion exchange resins are advantageously used, particularly crosslinked polystyrene with iminodiacetic acid functionality (commercially available as Amberlite 718). The chelating resin employed should be in a neutralized form. The sodium form of a material sold under the trade designation Chelex 100 by BIO-Rad Laboratories is also useful when properly prepared and intimately contacted with the membrane as described in Ambrus, which is hereby incorporated by reference.

Among other chelating agents that can be immobilized in the porous outer structure of the membrane are iminoacetic acid derivatives (such as ethylene diamine tetraacetic acid ("EDTA") and diethylene triamine pentaacetic acid ("DTPA")) and dithiocarbamate derivatives. When the molecular weight of a chelating agent is itself less than 50,000, the chelating agent should be immobilized on a macromolecular structure, e.g., silica gel, dextran, or the like, so that diffusion of the chelator to the blood side of the membrane is prevented.

As indicated above, Amberlite 718 is a preferred chelating material. Amberlite 718 has a high selectivity for lead versus calcium. Binding of calcium, however, can become significant when calcium is present

in a much higher concentration than lead, as in the situation where lead from bone tissue is being removed from blood. To avoid depletion of calcium, the Amberlite 718 resin is preferably equilibrated with an aqueous solution containing calcium and sodium at physiological concentrations. In some cases, it is advantageous to equilibrate the Amberlite 718 with another metal for which Amberlite 718 has a stability constant greater than that for calcium. Zinc is one such metal, and optimally, the Amberlite 718 is equilibrated with a mixture of zinc and calcium to prevent calcium depletion from blood.

The use of an anisotropic membranes, as described above, has a number of advantages, including the advantage of preventing contact between the solid resin and the formed elements of the blood.

It is preferable that the membrane be used in a tubular form, preferably with an inner diameter of about 200 μm and an outer diameter of about 300 μm, in order (a) to ensure a large contact surface area and (b) to facilitate the maintenance of appropriate flow velocities and flow distribution of blood over the membrane surface. Velocities that are too high, even locally, can result in excessive damage to the blood, especially to the circulating platelets. On the other hand, velocities that are too low tend to reduce the efficiency of heavy metal removal and prolong the time needed for treatment. In general, velocities known to the blood processing art, e.g., those used in hemodialysis, are suitable for use in the process of the invention.

Anisotropic membranes, i.e. those having a very thin barrier layer in contact with the blood and a more porous substructure as a support, are particularly useful in the process of the invention, because they allow the

chelating resin to be brought into intimate contact with the thin barrier membrane, impregnated well into the more grossly porous substructure of the membrane.

To facilitate this packing, the chelating resin is preferably reduced to a paste or slurry which can be packed closely around the exteriors of tightly packed membrane fibers in parallel with one another.

The nominal molecular weight retention values of a membrane which are referred to herein are known in the art to be appropriately determined with dilute solutions of standard materials, for example, proteins of known molecular weights.

The system, once it reaches equilibrium, is virtually free of any liquid flow through the membrane. Depending on the relative moisture content of the chelate-bearing substance at start up, liquid will seep rapidly from the blood to the chelate side. Thereafter, the liquid primarily serves to provide a diffusion path for the heavy metal through the membrane structure to the chelation sites. The membrane structure itself does not function as an ultrafilter but only as a "diffusion barrier", allowing heavy metal diffusion through the barrier layer and preventing diffusion of the larger blood components.

As indicated above, suitable ranges of nominal molecular weight retention values are from about 10,000 to about 50,000, preferably about 30,000. The nominal molecular weight retention value is preferably selected so as to prevent any loss of essential blood constituents but so as to permit diffusion of the heavy metals at issue.

Further details regarding this preferred method of removing heavy metals from blood are set forth in Ambrus, which is hereby incorporated by reference.

In many instances, it is possible to reduce the concentration of heavy metal in the subject's blood to levels below which treatment is mandated within a few hours. However, as indicated above, it may be desirable to reduce this level further so as to favor transfer of the heavy metal in the bone tissue to the blood when the blood is recontacted with the bone subsequent to extracorporeal circulation. Where the above-described, preferred anisotropic membrane and metal-capturing material is used, this can be done, for example, by increasing the surface over which the blood contacts the anisotropic membrane's barrier side or by increasing the time that the subject's blood is being extracorporeally circulated. Alternatively, the process can be carried out for a sufficiently long time so that the concentration of heavy metal in the subject's blood is significantly less that the level at which treatment is mandated. Furthermore, depending on the nature of the heavy metal being removed from the bone and the physiological conditions of the subject, establishing a new equilibrium between the subject's bone and blood may take days, weeks, or even months to achieve. Therefore, repeating the process of the present invention over these time frames may be advantageous. For example, the method of the present invention can be carried out at some first time T1 (for some duration $\Delta T1$ (e.g., 1 hour to 3 hours)) and again at some second time T2 (for some duration $\Delta T2$ (e.g., 1 hour to 3 hours)), where the first time T1 and second time T2 are temporally separated by more than about one month (e.g., T2-T1 is greater than one month,

greater than 1.5 months, greater than 2 months, and/or greater than 6 months).

Although the invention has been described in detail for the purpose of illustration, it is understood that such detail is solely for that purpose, and variations can be made therein by those skilled in the art without departing from the spirit and scope of the invention which is defined by the following claims.

WHAT IS CLAIMED:

1. A method for removing heavy metal from a subject's bone tissue, said method comprising:

contacting a subject's bone tissue with the subject's blood under conditions effective to transfer at least some of the heavy metal in the subject's bone tissue to the blood;

extracorporeally circulating the subject's blood; and

removing at least a portion of the heavy metal from the subject's blood while the blood is being extracorporeally circulated.

2. A method according to claim 1, wherein said removing at least a portion of the heavy metal from the blood comprises:

passing the blood along a retentive barrier side of an anisotropic membrane and, while preventing substantial flow of blood through the membrane, allowing the heavy metal to diffuse through the membrane into contact with a metal-capturing material capable of capturing the heavy metal.

3. A method according to claim 2, wherein the metal-capturing material is in a closed container.

4. A method according to claim 3, wherein the closed container, on being initially filled with liquid, prevents flow of blood through the anisotropic membrane.

5. A method according to claim 2, wherein the metal-capturing material is at least partially

immobilized in porous substance at the opposite side of the anisotropic membrane from the blood.

6. A method according to claim 2, wherein the metal-capturing material is an organic chelating agent.

7. A method according to claim 6, wherein the organic chelating agent a multi-valent, carboxylate salt.

8. A method according to claim 6, wherein the metal-capturing material is a finely-divided, multi-valent carboxylate salt-bearing resin.

9. A method according to claim 2, wherein the membrane has a maximum nominal molecular weight retention value below about 50,000.

10. A method according to claim 9, wherein the membrane has a maximum nominal molecular weight retention value of about 30,000.

11. A method according to claim 2, wherein the anisotropic membrane has a surface in the form of a plurality of tubes and wherein the metal-capturing material is disposed around the plurality of tubes.

12. A method according to claim 1, wherein the heavy metal is selected from a group consisting lead, cadmium, and combinations thereof.

13. A method according to claim 12, wherein the heavy metal is lead.

14. A method according to claim 1, wherein the subject has a blood concentration of lead below a level which would mandate removal of lead from the blood.

15. A method according to claim 1, wherein the heavy metal is lead and wherein the subject is a human adult and has a blood concentration of the lead below 45 μg/dL.

16. A method according to claim 1, wherein the heavy metal is lead and wherein the subject is a human adult and has a blood concentration of the lead below 35 μg/dL.

17. A method according to claim 1, wherein the heavy metal is lead and wherein the subject is a child and has a blood concentration of the lead below 15 μg/dL.

18. A method according to claim 1, wherein the heavy metal in the subject's bone tissue is the result of the subject's exposure to heavy metal over a prolonged period of time.

19. A method according to claim 1, wherein the heavy metal in the subject's bone tissue is the result of the subject's exposure to heavy metal more than ten years prior to the practice of said method.

20. A method according to claim 1, wherein the heavy metal is lead and wherein the subject's blood contains lead having a $^{207}Pb/^{206}Pb$ ratio greater than 110% of the $^{207}Pb/^{206}Pb$ ratio in the subject's exogenous environment.

21. A method according to claim 1, wherein the heavy metal is lead and wherein the subject's blood contains lead having a $^{208}Pb/^{206}Pb$ ratio greater than 110% of the $^{208}Pb/^{206}Pb$ ratio in the subjects exogenous environment.

22. A method for removing heavy metal from a subject's bone tissue comprising:

carrying out a method according to claim 1 at a first time, and

carrying out a method according to claim 1 at a second time, wherein the first time and the second time are temporally separated by more than one month.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/11420

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) :A61M 1/38, 37/00; B01D 15/08
US CL :Please See Extra Sheet.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 210/634, 638, 645, 646, 647, 649, 650, 651, 912; 435/2; 604/4.01, 5.04

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 4,612,122 A (AMBRUS et al) 16 September 1986 (12.09.86), see entire document.	1-22
Y	US 5,753,227 A (STRAHILEVITZ) 19 May 1998 (19.05.98), see entire document.	1-22

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 12 JUNE 2000	Date of mailing of the international search report 03 AUG 2000
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer SUN UK KIM DEBORAH THOMAS PARALEGAL SPECIALIST Telephone No. (703) 308-0661

Form PCT/ISA/210 (second sheet) (July 1998)★

INTERNATIONAL SEARCH REPORT

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A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

210/634, 638, 645, 646, 647, 649, 650, 651, 912; 435/2; 604/4.01, 5.04